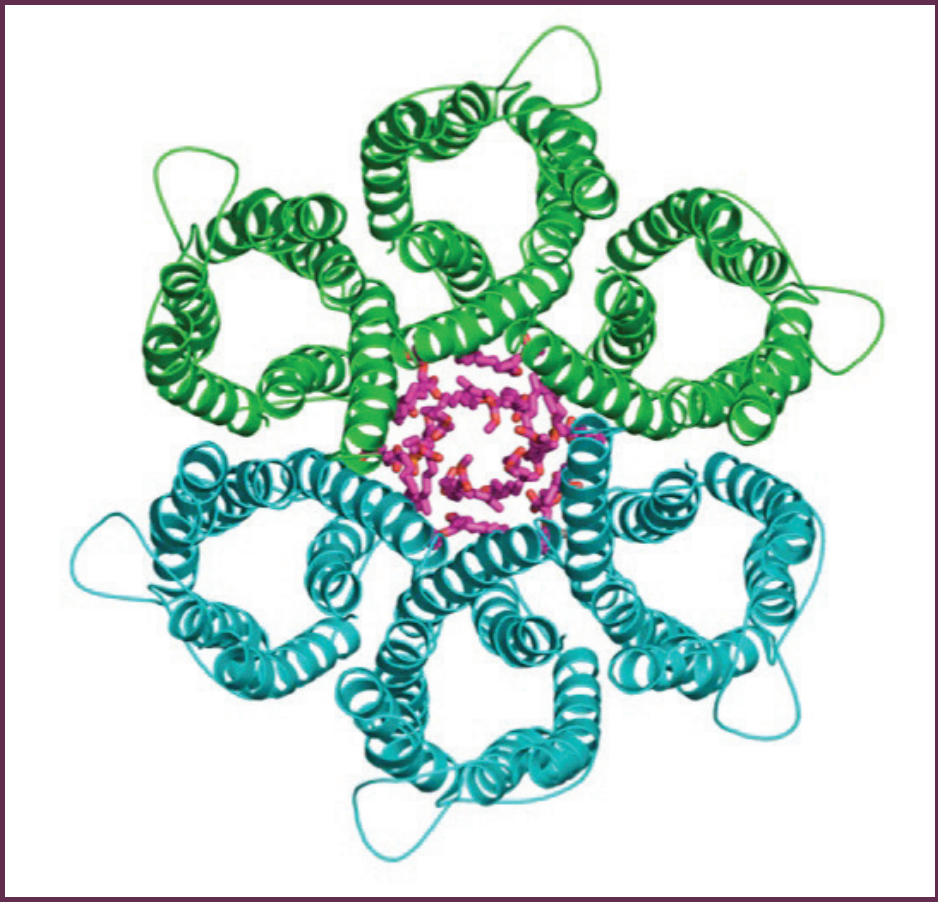

Helicobacter pylori (*H. pylori*) is a type of bacteria that causes stomach ulcers and can also cause stomach cancer. Shown here is the structure of the urea channel of *H. pylori*, which is essential for the bacterium to survive in the acidic stomach environment. The complex is made up of six subunits (in green and blue), making up six individual urea channels surrounding a lipid core (in purple). Having detailed information about essential structures of pathogens like *H. pylori* can aid in identifying targets for new treatments.

Image courtesy of Dr. Hartmut Luecke, University of California, Irvine and Dr. George Sachs, University of California, Los Angeles. Adapted by permission from Macmillan Publishers Ltd: Nature, Strugatsky, et al. Structure of the proton-gated urea channel from the gastric pathogen Helicobacter pylori*. 493: 255-258, copyright 2013.*

Digestive Diseases and Nutrition

Digestive diseases are among the leading causes of doctor visits, hospitalizations, and disability in the United States each year. These conditions span a wide spectrum of disorders that affect the gastrointestinal (GI) tract, liver, gallbladder, and pancreas, as well as obesity and other nutrition-related disorders. In 2004, the latest date for which reliable statistics are available, more than 35 percent of all emergency and outpatient hospital visits—some 100 million—were associated with a diagnosis of a digestive disease.[1] While some digestive diseases are common and others quite rare, collectively, they exact a significant toll on public health in terms of their effects on quality of life, years lost due to premature death, and costs associated with hospitalization and pharmaceutical and surgical interventions. To reduce the public health burden associated with digestive diseases, NIDDK-supported scientists are vigorously pursuing research to better understand how widespread these diseases are across the United States and in specific population groups, to identify the causes of these diseases and how they progress, and to test new interventions for prevention and treatment of these costly diseases, including drugs, surgery, and behavior modification.

Inflammatory bowel diseases (IBD), which include Crohn's disease and ulcerative colitis, are marked by destructive inflammation in the intestinal tract, leading to rectal bleeding, diarrhea, nutritional deficiencies, and other serious complications. These diseases often strike early in life, with a peak age of onset in adolescence or young adulthood. Treatment may require surgery, including removal of the affected region of the intestine. Scientists are investigating the complex interactions among the genetic, environmental, immune, microbial, and cellular factors that contribute to, or protect against, the development of IBD. The continued discovery of predisposing genetic variations, potential autoimmune and microbial influences, and new methods to repair damaged intestinal tissue will help catalyze the design of novel therapeutic strategies. Research on controlling intestinal inflammation has potential benefits not only for patients with IBD, but also for those at risk of developing colorectal cancer.

Diseases of the stomach and intestines include some of the most common digestive diseases, such as peptic ulcer disease, which is typically caused by an infection with the bacterium *Helicobacter pylori*, or use of non-steroidal anti-inflammatory drugs. Stomach and intestinal disorders also include functional bowel disorders, which result in symptoms of abdominal pain and altered bowel habits. For example, irritable bowel syndrome (IBS) causes pain and constipation or diarrhea. IBS more frequently affects women, who may display a different range of symptoms and respond differently from men to pharmacologic treatments for the disease. While diet and stress contribute to this disorder, its underlying causes are unknown. Gastroesophageal reflux disease, in which stomach acids rise up into the esophagus, is a common functional bowel disorder that can lead to a condition known as Barrett's esophagus. This condition, in which cells lining the esophagus turn into an intestinal type of cell, is associated with a heightened risk of esophageal cancer, which is one of the cancer types still on the rise in the United States. Gastroparesis

[1] *Everhart JE, editor. The burden of digestive diseases in the United States. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases. Washington, DC: U.S. Government Printing Office, 2008; NIH Publication No. 09-6443.*

is another functional bowel disorder, which is characterized by delayed emptying of food from the stomach, resulting in nausea, vomiting, and abdominal discomfort. While many cases of gastroparesis are of unknown origin, a common cause is diabetes, which is thought to damage nerves leading to the stomach and controlling movement of food. Fecal incontinence, or impaired bowel control, is another bowel disorder that poses a major public health burden. Although fecal incontinence is more common in older adults, it can affect people of any age. Because it is difficult to talk about, many people suffer without seeking professional treatment for this surprisingly prevalent condition. Researchers thus aim both to examine barriers to addressing fecal incontinence, and to develop improved treatment strategies.

Some digestive diseases can be triggered by the body's reaction to certain foods. For example, in individuals with celiac disease, the immune system reacts to the protein gluten—a component of wheat, barley, and rye—and damages the small intestine. This damage interferes with the ability of the intestine to absorb nutrients from foods and can result in chronic diarrhea, bloating, anemia, and, in children, slower growth and short stature. The only current treatment for celiac disease is maintenance of a strict gluten-free diet, which is difficult for many people. The greater challenge now facing patients and their health care providers is to improve methods capable of diagnosing celiac disease early, before damage occurs or other conditions develop. Recent and continued advances in the understanding of genes that predispose individuals to develop celiac disease may contribute to improved diagnosis in the future through genetic-based screening.

The microorganisms that inhabit the GI tract are important factors in maintaining or tipping the balance between digestive health and disease. These microbes can affect long-term health and nutritional status in some surprising ways, depending on their interactions with each other, with intestinal cells, and with nutrients ingested by their human host. Scientists are gaining insights into the ways these GI microorganisms influence the development and function of the digestive tract and other systems throughout the body, such as those with immune and metabolic functions, as well as how the composition of the GI microbe community changes with factors such as age, geography, diet, and antibiotic usage.

The exocrine pancreas, which secretes enzymes required for digestion, is vulnerable to disorders such as acute and chronic pancreatitis, and their complications. Common causes of pancreatitis include gallstones, heavy alcohol use, inherited genetic factors, and drugs. In all forms of pancreatitis, digestive enzymes attack the pancreas from within, causing inflammation, loss of function, and severe pain. Research has elucidated genetic and other factors contributing to pancreatitis that may lead to ways to treat or prevent this disorder.

The liver is an organ within the digestive system that performs many critical metabolic functions, including processing and distribution of nutrients such as fats. When the liver is functionally compromised by disease, serious adverse effects on health can occur, which sometimes leads to complete liver failure. Some liver diseases primarily affect children, such as biliary atresia (a progressive inflammatory liver disease), while others generally affect adults, such as a form of nonalcoholic fatty liver disease (NAFLD) or nonalcoholic steatohepatitis (NASH). In recent years, however, NAFLD has been increasingly diagnosed in children in the United States as well, concurrent with rising overweight and obesity. While some forms of liver disease are caused by viral infection such as hepatitis B and C, or by genetic mutations such as alpha-1-antitrypsin deficiency, others arise from diverse factors such as autoimmune reactions, drug toxicity, and other triggers, some of which are unknown. Many liver diseases, such as chronic hepatitis B and C, place individuals at elevated risk for developing liver cancer. A healthy liver is necessary for life, and the only treatment for end-stage liver disease is a liver transplant. Because the number of livers available from deceased donors is limited, research is critical to identify liver disease early, find methods to preserve liver function in people with liver disease, and develop and further study new treatment options, including transplants performed with liver tissue from living donors.

The number of Americans who are overweight or obese has risen dramatically in recent decades and is now at epidemic levels. Obesity is associated with numerous diseases, including type 2 diabetes, heart disease, and cancer. Multiple factors contribute to obesity. As scientists elucidate the molecular, genetic, microbial, and environmental factors that influence appetite, metabolism, and energy storage, they are identifying potential avenues for the development of new intervention strategies to promote safe, long-term weight loss. In addition to new pharmacologic interventions for obesity that may arise from research, existing bariatric surgical techniques are being evaluated for their long-term impacts on weight loss and well-being. Investigators are also continuing research to help people achieve healthy lifestyles that include physical activity and improved diet. (Additional information on NIDDK-supported research endeavors focusing on obesity is provided in the Obesity chapter.)

Other nutrition-related disorders under investigation involve specific, inherited alterations in nutrient metabolism. NIDDK-supported research has enhanced knowledge of how these nutritional disorders develop, and how they can best be treated.

NUTRITION AND DISEASE

Connecting Food, Genes, and Rare Metabolic Diseases: Researchers have mapped out an intricate network of genes that connect metabolism, responses to different foods, and rare metabolic diseases in a study of tiny worms. Seeking to understand the effects of different diets on gene activity, the research team selected a model organism that would enable them to carry out experiments not possible in humans. With a miniature digestive system, a transparent body, and a genome readily amenable to manipulation, the small (one millimeter) worm, *C. elegans*, affords numerous advantages for discovering genes that can then be analyzed in people. Worms will also eat different types of food in the laboratory, allowing examination of responses to different diets.

As a starting point for their study, the researchers found a gene with activity that ramped up or diminished depending upon what the worms ate; this gene appears to be involved in breaking down certain amino acids, which are components of proteins. They attached this gene to a fluorescent marker that, reflecting the gene's activity, would glow to a greater or lesser extent based on the type of food eaten. Using fluorescence as a dietary sensor (visible through the transparent worms), along with other experimental techniques, the researchers were able to identify over 180 other genes that worked with the first one in a vast network to respond to dietary nutrients and other aspects of metabolism. Some of these genes encode enzymes important in breaking down various components of food, and some of the genes appear regulatory in nature, modulating the extent to which other genes are activated or shut down. Applying their new knowledge to gain insight into human metabolism, the researchers found that many of the worm genes had counterparts in humans. This network may thus mirror similar interconnections among human genes, elucidating pathways by which the body senses different dietary nutrients or other metabolic signals, and modulates gene activity to be able to digest and process the nutrients or respond in other ways as needed. Moreover, several of these human genes are known to be associated with rare diseases. Collectively referred to as inborn diseases of amino acid metabolism, these diseases are caused by mutations in the genes and are marked by incomplete breakdown of certain amino acids; treatments involve specific dietary modifications. Thus, in addition to advancing understanding of metabolism and response to diet, this study may also lead to new avenues for therapeutic development to improve the health of people with inborn metabolic diseases.

Watson E, MacNeil LT, Arda HE, Zhu LJ, and Walhout AJM. Integration of metabolic and gene regulatory networks modulates the C. elegans *dietary response. Cell 153:253-266, 2013.*

GUT MICROBES IN HEALTH AND DISEASE

Gut Microbes Contribute to Severe Malnutrition in Young Malawian Twins: A study of young twin pairs in Malawi has shown that gut microbes may play an important role in causing severe malnutrition in children that persists in spite of nutritional interventions. Malnutrition is the top cause of child mortality in the world. Childhood malnutrition is common in some African countries like Malawi, where the diet is deficient in protein and the rate of infant mortality is one of the highest in the world. A type of severe malnutrition called kwashiorkor is thought to result from a combination of inadequate nutrient intake, particularly of protein, and other environmental factors. Although it is not fully understood why some children develop kwashiorkor, evidence from past studies has indicated a possible role in early development for the trillions of microbes that reside in the human gut. A group of researchers from the United States, Malawi, and the U.K. conducted a study of identical and fraternal twins born in Malawi to identify any links between gut microbes and severe malnutrition. They collected fecal samples every two weeks from pairs of twins with malnutrition under three years of age—before, during, and after they were given the standard treatment of ready-to-use therapeutic foods or supplements. In some cases, both twins became well-nourished; in others, one or both of the twins relapsed and again became malnourished after the nutritional therapy. The scientists concentrated on those pairs—similar in genetics, diet, and environment—in which one twin was well-nourished after treatment, while the other developed kwashiorkor. They analyzed the fecal samples to detect DNA from gut microbes, and found that different gut microbes were present in the well-nourished compared to the malnourished twin.

To see if the different microbes were affecting the nutritional state of the children, the scientists transplanted fecal matter from these twins into the guts of mice raised previously under sterile conditions. Then they fed the mice a diet based on foods eaten in Malawi prior to a two-week treatment with the ready-to-use therapeutic food, followed by resumption of the Malawian diet. The mice transplanted with the malnourished twins' microbes lost much more weight than the mice transplanted with microbes from the well-nourished twins. Similar to the human twins, mice transplanted with the malnourished twins' fecal samples harbored different types of bacteria, some of which have been linked to bowel disease, from those transplanted with material from the well-nourished twins. After the nutritional therapy, the mice with the malnourished twins' microbes showed a shift to more healthy types of bacteria along with indicators of improved nutrition and metabolic function. However, after being put back on the Malawian diet for only four weeks, these indicators fell back to pre-treatment levels. This study's finding—that the combination of dietary deficiency with a particular gut microbial profile causes severe malnutrition—has far-reaching implications for developing sustainable interventions for childhood malnutrition. For example, biomarkers of microbial metabolism may need to be taken into account for designing effective nutritional interventions in some individuals. These and other insights will be helpful in developing more effective approaches to treating and preventing the huge global challenge of severe malnutrition in children.

Smith MI, Yatsunenko T, Manary MJ, et al. Gut microbiomes of Malawian twin pairs discordant for kwashiorkor. Science 339: 548-554, 2013.

Gut Microbes from People Can Transmit Obese or Lean Body Types to Mice: A study in mice has found that gut microbes obtained from obese or lean people, within certain dietary contexts, can transmit obesity or leanness to mice in the lab. While there is ample evidence that genetics and other factors play an important role in the development of obesity, there is also evidence that the community of bacteria living in the gut and their collective bacterial genomes, or "gut microbiome," may affect and reflect a person's health and nutritional status. For example, recent research raised the possibility that differences in the gut microbiome may explain why twins with identical genetic makeups can have very different disease and nutritional states.

A group of researchers were interested in exploring this idea using sets of identical twins who were "discordant" for obesity, which means one twin was obese while the other was not. Previous research with such discordant twins has shown that obesity is associated with changes in the types of bacteria in the gut; however, it is unclear whether these changes in the gut microbiome actually contribute to the development of obesity. To examine this possibility, scientists transferred the gut microbiomes from twins discordant for obesity into mice previously raised in sterile conditions and initially free of any gut microbes. Even though all mice were fed the same diet, only the mice that received the obese twin's microbiome gained weight, while the mice that received gut microbes from the lean twin did not.

Knowing that mice often share gut microbes with their cage mates, the scientists housed the "lean" mice—those inhabited by the lean human twin's gut microbes—together with the now "obese" mice—those with the obese twin's gut microbes—to see if the microbes from one set of mice would spread to, and affect, the other set of mice. Under these conditions, the weights of the lean mice did not change, but after several days the obese mice lost a significant amount of weight and began to harbor the same types of bacteria that were in the lean mice. This finding suggested that the microbiome from the lean mice, along with its lean-promoting effects, was being transferred to the obese mice, but not *vice versa*.

When the scientists compared the microbiome from the lean mice to that of the obese mice, they found differences in genes that regulate metabolism, including the metabolism of certain amino acids (components of proteins) and effects on fats and starches, suggesting that metabolic changes are responsible for the microbiome's effects on weight. However, the protective effects of the lean twin's microbiome were only seen when the mice were fed a healthy diet with high amounts of fruits and vegetables and low amounts of saturated fat, meaning that changes in weight were not dependent on the microbiome alone, but were also dependent on diet.

These studies provide convincing evidence that the gut microbiome, in conjunction with diet, can strongly affect the ability to gain or lose weight in mice, and may lead to insights into the role of the gut microbiome in regulating weight in humans.

Ridaura VK, Faith JJ, Rey FE, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science 341: 1241214, 2013.

Molecule Critical for Gut Pathogen Survival Visualized: Researchers have revealed the structure of a protein complex important for the survival of a species of bacteria that causes gastric ulcers and other gastrointestinal diseases. *Helicobacter pylori* (*H. pylori*) is a species of bacteria that is commonly found living in the human stomach. It is thought to damage the mucus coating that protects the stomach lining, exposing it to powerful digestive acids. This can result in gastric ulcers, which are painful sores that can be accompanied by such symptoms as weight loss, vomiting, and bleeding. Antibiotics are typically used to treat *H. pylori* infections, but they are not always successful and can cause the bacterium to develop antibiotic resistance. This bacterium may have an Achilles' heel, however, as it can only survive the harsh environment of the stomach by neutralizing the potent stomach acids that would otherwise digest the bacterium. It does this by taking up a molecule called urea through a selective opening, a "channel," on the surface of the bacterium. Once inside, the urea is converted into ammonia, which is then used to neutralize the stomach acid. This channel is an ideal target for drugs that can block the entry of urea into the bacterium, thereby threatening its survival, and determining the three-dimensional structure of the channel is necessary before drugs can be designed efficiently.

To obtain the structure, a group of scientists undertook the extremely difficult task of collecting large amounts of the protein complex that creates the channel, purifying it, and condensing it into crystals. They were then able to deduce the structure of the complex by analyzing the pattern made by X-rays after they passed through the crystals, a technique called

X-ray diffraction, the same approach that was used to discover the double helix structure of DNA. The result of the study was the generation of an elegant, three-dimensional model of the channel that *H. pylori* uses to stay alive inside the stomach. The scientists were then able to identify the precise segment that is required for urea entry by changing parts of the channel and testing its ability to take up urea. Now that the detailed structure and critical features of the channel have been defined, scientists can design specific drugs that will block its function, like designing a plug to fit into a hole, thereby preventing the entry of urea into *H. pylori* and, ultimately, impeding its survival in the stomach. This would provide a new strategy to combat this pathogen and open the possibility of new treatments for the prevention of ulcers.

Strugatsky D, McNulty R, Munson K, et al. Structure of the proton-gated urea channel from the gastric pathogen Helicobacter pylori. *Nature 493: 255-258, 2013.*

People and Their Gut Microbes Are Life-long Partners: Scientists have found that the types of bacteria in our gut change very little throughout our lives. The human microbiome, or the collection of microbes present in the body, includes trillions of bacteria living in our gastrointestinal tract. There is evidence that the composition of the gut microbiome can affect human health. One common approach to study the gut microbiome is to sequence the DNA of the bacteria living there, but this method has been limited by the sensitivity and accuracy of the DNA sequencing technique, which had difficulty discerning between individual types or strains of bacteria and errors in the sequencing process. To overcome this limitation, a group of researchers developed a new way to sequence the DNA of gut bacteria that improves the accuracy to a point where the scientists can differentiate between individual bacterial strains. Using this method to sequence DNA contained in stool samples from healthy adults over time, the scientists found that the relative amount of each bacterial strain changes very little over several years. In other words, the demographics of the gut microbial community are likely stable in healthy individuals for decades or longer. The researchers also compared the microbial communities of family members and found them to be much more similar than the microbiota of unrelated people. This finding suggests that family members are colonized by similar bacteria through exposure to a shared environment and that the gut microbiome is established relatively early in a person's life—probably within a few years after birth.

The researchers did find one situation that will disrupt the stability of the microbiome, however: when someone undergoes a drastic change in diet that causes substantial weight loss. A group of volunteers consumed a calorie-restricted liquid diet for several months and subsequently lost about 10 percent of their body weight. The stability of the gut microbiome quickly broke down under these conditions, and the individuals whose weight fluctuated the most also had the least stable microbiomes. Overall, these studies suggest that the types of bacteria in the gut microbiome will remain relatively constant throughout a person's life, though this stability is affected by changes in diet and weight.

This points to the gut microbiome as a potential predictor of an individual's state of health, such that changes in the microbiome could be used in the future as markers for overall well-being and disease development.

Faith JJ, Guruge JL, Charbonneau M, et al. The long-term stability of the human gut microbiota. Science 341: 1237439, 2013.

IRRITABLE BOWEL SYNDROME

Brief Psychological and Educational Therapy Improves Symptoms of Irritable Bowel Syndrome: Researchers have found that patients with irritable bowel syndrome (IBS) show an improvement in symptoms following a short course of group therapy involving psychological and educational approaches. IBS is a collection of symptoms, including abdominal pain or discomfort (such as cramping), along with diarrhea, constipation, or both. A primary reason why IBS is difficult to treat is that its exact causes are not well understood, although it is believed to have both

physical and mental origins. One possible cause is a problem with communication between the brain and the gut, which could lead to changes in bowel habits. Based on this likely mind-body connection, some successful treatment of IBS has been achieved using psychological counseling and education-based therapies to help patients control the activity of their own nervous systems and gastrointestinal tracts, but variable results, along with cost issues, unavailability of trained clinicians, and a general preference for pharmaceutical remedies, have hindered the implementation of psychological therapy as a standard of treatment.

To determine if a combination of psychological and educational therapy can lead to a sustained improvement in IBS symptoms and an increase in the quality of life for patients, a team of scientists performed a clinical trial where IBS patients in the intervention group attended a five-week series of two-hour group classes co-led by a gastroenterologist and therapist to promote self-efficacy and teach relaxation techniques. As a basis for comparison, a control group of patients was monitored while on the waiting list for the group classes. Patients who attended the group classes learned about the linkage between emotions, stress, and abdominal symptoms with an emphasis on their ability to control the activity of their own bodies. They were also taught about the connection between mood, stress, and GI symptoms, and the difference between ineffective coping styles, such as panicking during moments of anxiety, and more effective responses, such as arriving at conscious, rational solutions during stressful situations. The classes also instructed patients on deep breathing techniques and progressive muscle relaxation, including homework assignments consisting of at least 15 minutes of relaxation exercises twice a day. During and after the trial, the class participants and control patients were asked to monitor and document their symptoms in relationship with their mood states, stressors, and diets. The results of this trial suggested that patients who underwent the group psychological and educational therapy had a reduction in IBS symptoms and a better quality of life, lasting for at least three months after the trial, than those who did not participate in the class. The therapy was particularly helpful for those individuals who had a low or average quality of life prior to starting the intervention, although it had less of an effect for those whose quality of life was higher at the beginning of the study. This study demonstrated an effective, low-cost method of treating IBS symptoms, especially for those with low or average health-related quality of life, and could pave the way for the adoption of such an approach as an alternative to, or a supplement for, pharmacological therapy.

Labus J, Gupta A, Gill HK, et al. Randomised clinical trial: symptoms of the irritable bowel syndrome are improved by a psycho-education group intervention. Aliment Pharmacol Ther 37: 304-315, 2013.

INFLAMMATORY BOWEL DISEASE

New Insights into the Mechanism of Intestinal Lining Repair: Researchers have discovered a new role for a protein called Wnt5a in the regeneration of damaged gut tissue. The human intestinal tract is prone to injury by conditions such as inflammatory bowel disease, where the lining of the gut is damaged as a result of chronic inflammation and irritation. An important component of the intestinal lining is a collection of specialized structures called "crypts," which are small depressions in the lining of the gut. At the base of these crypts are intestinal stem cells, which replenish the injured lining by proliferating and maturing into specific cell types needed to replace the damaged tissue.

Intestinal crypts are damaged during injury to the lining, but how these vital structures are replaced is unclear. A group of scientists set out to address this question using a mouse model of simulated intestinal injury by surgically removing some of the crypts in the mouse's gut, then examining the intestinal tissue both within and nearby the damaged site. They found that cells from intact crypts near the damaged site will migrate into the wound and form a series of shallow channels, which eventually subdivide into new crypts to replace the damaged ones. When the scientists

examined some of the cells in these channels, they found a high abundance of a protein called Wnt5a, which suggested that it might be important for crypt regeneration. To test this idea, the researchers looked at intestinal tissue healing in a mouse model that is missing Wnt5a from its intestinal cells. When the intestinal lining of these mice was injured, no new crypts were formed, confirming that Wnt5a was necessary for the renewal of these structures. The scientists were then interested in exactly how Wnt5a was guiding the formation of new crypts, so they looked at changes in intestinal cells in culture upon exposure to this protein. They found that the cells stopped proliferating and formed small channels, similar to what happened in the mouse model. Further analysis of the cells exposed to Wnt5a showed that this protein activates a pathway that is known to stop cell proliferation, providing insight into the mechanism by which Wnt5a supports crypt regeneration. This research sheds light on how intestinal tissue is repaired after injury, and could lead to new ways to help facilitate the regeneration of intestinal cells that are damaged in diseases like inflammatory bowel disease.

Miyoshi H, Ajima R, Luo CT, Yamaguchi TP, and Stappenbeck TS. Wnt5a potentiates TGF-β signaling to promote colonic crypt regeneration after tissue injury. Science 338: 108-113, 2012.

Intestinal Inflammation Works Through Microbes To Raise Cancer Risk: Researchers have uncovered a surprising interaction between intestinal inflammation and a type of bacteria that promotes colorectal cancer (CRC). Chronic intestinal inflammation is considered a risk factor for CRC, although the process by which it increases risk is unclear. The human intestine is also home to trillions of microbes, which reside near the intestinal cells affected by inflammation and cancer. One research group questioned whether these microbes were innocent bystanders, or if they played a role in the transition from inflammation to cancer within the intestine. First, they sequenced genetic material from bacteria in intestinal and stool samples taken from a mouse model of intestinal inflammation that was genetically programmed to develop colitis (colonic inflammation). They also analyzed samples from control, non-altered mice. The sequencing showed that while the total numbers of intestinal bacteria were similar, the types of microbes found inside the colon differed in mice predisposed to colitis compared to controls. One type of bacteria in particular that was more abundant in the mice with colitis was *Escherichia coli* (*E. coli*), which is also more plentiful in the colons of humans with inflammatory bowel disease (IBD) and CRC. When the mice were given a chemical carcinogen, only those predisposed to colitis developed cancer. Next, the researchers inoculated mice raised under germ-free conditions with a single type of intestinal bacteria—either *E. coli* or another type of bacteria called *Enterococcus faecalis* (*E. faecalis*). The colitis-prone mice inoculated with *E. coli* and given the chemical carcinogen developed an invasive form of colon tumors, while the mice inoculated with *E. faecalis* and given the carcinogen were less likely to develop invasive tumors. Searching through the *E. coli* bacterium's genome, the researchers identified a gene in some bacterial strains coding for a protein that damages DNA. They found that a strain of *E. coli* with this toxic gene was more likely to damage DNA in an intestinal cell line compared to another *E. coli* strain lacking this gene. Likewise, infection of the colitis-prone mice with this toxic strain of *E. coli* resulted in more numerous and invasive tumors than those in mice infected with the less-toxic strain. The researchers also found this strain of *E. coli* with the DNA-toxic gene in samples taken from patients with IBD and CRC. This study provides evidence that certain types of intestinal microbes are key players in the complex transformation of colon cells from an inflamed to a cancerous state. Future research will likely focus on other bacterial strains that may contribute to this progression from intestinal inflammation to CRC.

Arthur JC, Perez-Chanona E, Mühlbauer M, et al. Intestinal inflammation targets cancer-inducing activity of the microbiota. Science 338: 120-123, 2012.

Particles Released from a Gut Microbe Protect Against Colitis: A team of scientists has discovered how a species of gut bacteria interacts with the immune system to suppress inflammatory bowel disease (IBD),

in a study in mice. IBD is the general name for the diseases, such as Crohn's disease and ulcerative colitis, that cause symptoms such as abdominal pain and diarrhea due to inflammation and irritation in the intestines. *Bacteroides fragilis* (*B. fragilis*), a species of bacteria that inhabits the human intestinal tract, can suppress inflammation in diseases like IBD. This type of bacteria makes a molecule called polysaccharide A, which not only protects the bacteria from the harsh environment of the intestine, but also activates a group of immune cells called regulatory T cells that inhibit inflammation by restraining the immune response. However, exactly how *B. fragilis* interacts with the immune system through this bacterial molecule was a mystery.

A group of researchers tackling this question found that polysaccharide A is released from cultures of *B. fragilis* in small spheres, called outer membrane vesicles (OMVs), which bud from the bacterial cells' outer coating. When given to mice orally, these OMVs prevented an experimental form of colitis. When OMVs were added to immune cells in laboratory culture, polysaccharide A was found to accumulate in a type of immune cell called a dendritic cell, which communicates with regulatory T cells to activate them. This is particularly important in the gut, because dendritic cells have the key role of sampling intestinal contents to coordinate T cell reactions. When OMVs were added to cultures containing both dendritic cells and T cells, there was an increase in the production of an anti-inflammatory chemical produced by the T cells. The researchers predicted that a dendritic cell protein called TLR2, which senses bacterial products, was necessary for this response. Indeed, when OMVs were added to dendritic cells that were lacking TLR2, the dendritic cells did not cause an increase in the anti-inflammatory chemical in T cells. In support of this, the scientists found several TLR2-dependent genes that were turned on in dendritic cells by polysaccharide A, including a gene called *Gadd45a*, which is required to promote T-cell response. Mice that were genetically manipulated to lack the *Gadd45a* gene were not protected against colitis when they were treated with OMVs, unlike the non-genetically manipulated mice. This study demonstrates a fascinating interaction on a molecular level between a gut microbe and the immune system of its mouse "host" that leads to protection against disease. More importantly, it opens the possibility for future research in humans that could potentially lead to new therapies for inflammatory bowel disease.

Shen Y, Giardino Torchia ML, Lawson GW, Karp CL, Ashwell JD, and Mazmanian SK. Outer membrane vesicles of a human commensal mediate immune regulation and disease protection. Cell Host Microbe 12: 509-520, 2012.

LIVER METABOLIC FUNCTION AND DISEASE

Tiny RNA Has Big Effects on Lipid Metabolism and Atherosclerosis: Scientists have shown that a small molecule, called "microRNA," plays a big role in lipid (fat) metabolism and related health conditions such as hyperlipidemia (elevated lipid levels in the blood) and atherosclerosis (clogging and hardening of the arteries). High levels of lipids circulating in the blood put individuals at higher risk for cardiovascular and metabolic disorders. Lipids are carried in the blood in packages called lipoproteins, which are assembled, mostly in the liver, with help from proteins such as the microsomal triglyceride transfer protein, or MTP. Researchers aimed to determine whether there were any regulators of MTP in the body that might be targets for lowering elevated blood lipids. MicroRNAs or "miRNAs" are small pieces of RNA that target another type of RNA, abbreviated mRNA (for messenger RNA), which is involved in protein production—ultimately, reducing the amount of protein made from the mRNA.

The research team looked at several miRNAs that could potentially regulate MTP and identified one—miR-30c—that reduced MTP activity, as well as decreased the concentration of lipoproteins secreted in a cell culture model. They found that miR-30c accomplishes this feat by binding to and degrading MTP mRNA. To test the miRNA's effects on physiological functions, they moved to an animal model—mice that were injected with a substance to boost miR-30c levels in the liver, in particular. After three weeks, the mice had reduced levels of MTP in the liver, as well

as cholesterol in the blood, reflecting that amounts of certain lipoproteins were also lowered. Surprisingly, the abundance of miR-30c not only inhibited the packaging of lipids into lipoproteins, but it also inhibited the synthesis of the lipids themselves, suggesting an even greater role for this miRNA in lipid metabolism. Mice with extra miR-30c in the liver also had fewer, smaller plaques in their arteries; large amounts of arterial plaques are a sign of atherosclerosis. The results of this study outline the key role of this specific miRNA in reducing lipid synthesis, secretion of specific lipoproteins, and, ultimately, atherosclerosis. These findings highlight the potential importance of agents that could boost or mimic the effects of this miRNA as a treatment for those at risk for cardiovascular and metabolic disorders.

Soh J, Iqbal J, Queiroz J, Fernandez-Hernando C, and Hussain MM. MicroRNA-30c reduces hyperlipidemia and atherosclerosis in mice by decreasing lipid synthesis and lipoprotein secretion. Nat Med 19: 892-900, 2013.

Cell Signaling Pathways Point to Top Notch Solution for Fatty Liver Disease: Scientists have identified a cell signaling pathway that may contribute to the development of fatty liver disease and may also point to therapeutic solutions. Obesity is associated with several metabolic diseases, including type 2 diabetes and nonalcoholic fatty liver disease, or "NAFLD," which is marked by fat accumulation in the liver. Treatment options for NAFLD are particularly limited. One mystery surrounding these obesity-associated metabolic diseases has been how some insulin-responsive pathways in liver cells, such as fat production, continue to function when insulin is elevated—ultimately, contributing to fatty liver—while others, such as suppression of glucose production, become resistant to the action of insulin. Researchers explored cell signaling pathways that could be responsible for the overproduction of fat in the liver associated with insulin resistance. They narrowed their search to a signaling pathway initiated at a receptor protein spanning the cell surface known as "Notch," which regulates some metabolic functions in the liver, but is also well-known for its role in early development. After feeding mice a high-fat diet, the team saw greater Notch activity in the liver, along with more fat deposition in the organ. However, when mice were genetically altered to lack a key factor in the liver needed for Notch signaling, their livers showed less fat accumulation than in the non-genetically altered mice. Similar results were observed when Notch signaling was again disabled, this time in mice with a defective Notch receptor protein. To gather more evidence for their theory, they also created a mouse model in which Notch signaling was active all the time. In this model, they observed fat accumulation in the liver even though the mice were given a more balanced (non-high-fat) diet. Using the expression of some genes that are typically affected by the Notch signaling pathway as a readout, the scientists determined that Notch signaling acts through a molecule within the cell called mTorc1 to increase fat production in the liver. If studies in humans show a similar role for Notch signaling, this could be an important therapeutic target to block in treating NAFLD associated with obesity. This finding is especially promising in light of the fact that agents to inhibit Notch signaling are already available and being tested in clinical trials against other diseases.

Pajvani UB, Quang L, Kangsamaksin T, Kitajewski J, Ginsberg HN, and Accili D. Inhibition of Notch uncouples Akt activation from hepatic lipid accumulation by decreasing mTorc1 stability. Nat Med 19: 1054-1061, 2013.

Treatment To Prevent Hepatitis C Recurrence After Liver Transplantation: A clinical trial conducted at U.S. liver transplant centers has shown that pre-treatment of patients infected with hepatitis C, using antiviral therapy to suppress the viral infection, can prevent recurrence of the infection once patients are transplanted with a healthy donor liver. Chronic hepatitis C is one of the major reasons for adult liver transplantation. Adult-to-adult living donor liver transplantation—removal of part of a living adult's healthy liver for transplantation into another adult with liver disease—is one option for patients whose livers have been damaged by hepatitis C. However, in patients who still have hepatitis C virus (HCV) circulating in their blood at the time of transplantation, the infection will inevitably recur and threaten the health of the transplanted organ, whether from a living or cadaveric donor. Previous small studies had suggested one strategy to prevent this is with treatment prior to transplant using antiviral drugs against

HCV—pegylated interferon and ribavirin—to suppress viral levels in the blood. But this antiviral treatment is less effective in individuals with some types of the virus and can cause side effects.

Researchers at seven U.S. transplant centers participating in the Adult-to-Adult Living Donor Liver Transplantation Cohort Study (A2ALL) performed the first randomized clinical trial assessing the efficacy and safety of antiviral therapy pretreatment to prevent hepatitis C recurrence after liver transplantation. Patients infected with some of the HCV types prevalent in the United States were recruited into treatment and control groups. Prior to transplant, the treatment groups were started on a low dose of peginterferon and ribavirin that steadily increased over the course of treatment, which lasted up to a maximum of 48 weeks. The researchers measured viral RNA levels to determine whether the virus was responding to treatment; an undetectable level of viral RNA in the blood after several weeks of treatment, before then after transplant, represented a response. They found that patients who were treated prior to transplant for at least 16 weeks were more likely to have undetectable levels of HCV after transplant than those with shorter treatments. Overall, a similar number of total adverse events occurred in the treated and control groups, although the number of adverse events per person and also incidence of the particular adverse event of bacterial infection were higher in the treated groups. This study provides clinical evidence that antiviral therapy prior to a liver transplant can prevent hepatitis C recurrence in some patients. This finding is particularly applicable to patients who are better able to tolerate an extended course of antiviral therapy and its potential side effects.

Everson GT, Terrault NA, Lok AS, et al. A randomized controlled trial of pretransplant antiviral therapy to prevent recurrence of hepatitis C after liver transplantation. Hepatology 57: 1752-1762, 2013.

Biliary Atresia Susceptibility Gene Identified in Patients: Scientists participating in NIDDK's Childhood Liver Disease Research and Education Network (ChiLDREN) have utilized patient samples and an animal model to identify a genetic deletion that may play a role in the development of biliary atresia. Biliary atresia (BA) is a life-threatening condition affecting newborn infants in which the bile ducts—tubes that carry bile from the liver to the gallbladder for storage and to the small intestine to aid fat digestion—become blocked and destroyed, leading to a back-up of bile and liver damage. Most infants with biliary atresia require surgery to survive and may later need a liver transplant as well. Although its causes are unknown, some have speculated that it results from a combination of environmental and genetic factors. A group of researchers at a site participating in the NIDDK's ChiLDREN Network aimed to build on past findings of an association between a region of the genome and BA by looking for a specific gene(s) within this region that confers increased disease risk. By comparing genetic sequences from patient blood samples at the Network site with healthy controls, they zeroed in on a small part of this genetic region that was deleted in the patients. In healthy individuals, this region contains only one gene, called *GPC1*, coding for a protein involved in organ development. To more directly observe the functional impact of this gene's deletion on biliary development, they turned to an animal model with an analogous form of the gene (*gpc1*) and similar biliary anatomy—the zebrafish. In this model, the research group could use molecular inhibitors to reduce activity of the fish's *gpc1* gene early in development, mimicking the deletion of this gene in the patients with BA. They observed defects in biliary anatomy in the fish larvae with reduced *gpc1*, but not in the control larvae. Applying this knowledge to the human disease, they looked for the GPC1 protein in liver samples from patients with BA and healthy controls. The livers of patients with BA showed lower levels of GPC1 compared to the livers from healthy controls. This study identifies *GPC1* as a new potential susceptibility gene that may be lacking in patients with BA and shows the mechanism in an animal model by which this gene deletion may result in human disease. The ChiLDREN Network has enabled studies of BA such as this one through collecting samples from a sufficient number of patients affected by this relatively rare disease.

Cui S, Leyva-Vega M, Tsai EA, et al. Evidence from human and zebrafish that GPC1 *is a biliary atresia susceptibility gene. Gastroenterology 144: 1107-1115, 2013.*

Drug-Induced Liver Injury Network

Drug-induced liver injury, though relatively uncommon in the United States, represents the leading cause of acute liver failure in the nation and the top reason for regulatory actions by the U.S. Food and Drug Administration against approved medications. Products on the market implicated in causing liver injury currently include several hundred prescription drugs, over-the-counter medications, herbal products, and dietary supplements. However, knowledge has been limited concerning the scope of this problem in the U.S. population, mechanisms by which susceptible individuals develop drug-induced liver injury, or how to definitively diagnose these cases. Testing of new drugs in pre-clinical and clinical trials before they are approved and reach the wider patient population does not always provide reliable information on their potential risk of causing liver injury. Diagnosis of drug-induced liver injury can also be complicated by similarities to other forms of liver disease and variability in responses across patients—currently, it is a "diagnosis of exclusion," made only after all other possible causes have been ruled out.

The NIDDK established the Drug-Induced Liver Injury Network (DILIN) in 2003 to collect and analyze cases of severe liver injury caused by prescription drugs, over-the-counter drugs, and alternative medicines, such as herbal products and supplements. Since that time, DILIN has collected more than 1,000 cases of liver toxicities due to these agents and made major contributions to characterizing potential mechanisms and disease processes; defining the clinical spectrum, natural history, and outcomes of liver injury that results from these agents; and aiding accurate diagnosis. For example, studies of data generated by DILIN have informed the use of assessment tools used for diagnosing drug-induced liver injury and have also characterized the pathological and clinical features, as well as outcomes resulting from this form of injury. Additionally, the Network has found that, over time, the number of enrolled cases of liver injury attributed to herbal and dietary supplements, particularly supplements used for bodybuilding, has steadily increased, now representing the second most commonly recorded class of liver toxicity-causing products in the Network. Analyses of Network data also yielded the surprising finding that some cases previously diagnosed by physicians as drug-induced liver injury in the United States were attributable instead to infection with the hepatitis E virus.

An additional benefit of DILIN came in 2012 in the form of a website called "LiverTox," which was developed by the NIDDK in conjunction with the NIH's National Library of Medicine. This website features sample cases of drug-induced liver injury based on the Network data, as well as a database with summaries of liver injury reports for a given drug or herbal/dietary supplement. The website serves as a public resource to aid health care providers in diagnosing, and investigators in studying, liver injury due to drugs and herbs/supplements. Additional information on LiverTox is available at: *http://livertox.nih.gov/*

In 2013, the NIDDK announced a new initiative to continue and expand the Network and the studies it enables. The new initiative will have several objectives for the renewed Network, including: to enhance enrollment of individuals who experienced drug-induced liver injury from diverse backgrounds and geographic distributions; attempt to enroll patients as early as possible after injury to better characterize disease processes; develop an accurate and user-friendly computer-based instrument for improved diagnosis; continue clinical and genetic analyses to determine the role of genetic variability in this form of liver injury; support studies of disease processes and potential means of prevention or treatment; and continue support for the LiverTox website as a resource for collecting additional cases from the medical community. The renewed Network will be composed of up to five clinical centers across the country with expertise in diagnosis and management of drug-induced liver injury and a data coordinating center. Once the new Network is formed, an invitation will be sent to all academic medical centers in the United States to identify and document cases of drug-induced liver injury at their sites, using the online case report system developed by the Network investigators, the NIDDK, and the NIH's National Library of Medicine. In this way, this unique Network will be strengthened in its efforts to yield new insights into diagnosis, treatment, and, ultimately, prevention of drug-induced liver injury.

Fecal Incontinence Workshop

On August 19-20, 2013, the NIDDK hosted a workshop, "Developing a Clinical Research Agenda for Fecal Incontinence," to address the gaps in clinical and basic research for this condition that affects nearly 18 million adults[1] and represents a significant personal and public health burden, with considerable economic cost. The speakers included a panel of experts in epidemiology, gastrointestinal physiology, gastroenterology, colorectal surgery, urogynecology, psychology, and behavioral medicine. During the workshop, the panel identified and discussed major issues in the diagnosis and treatment of fecal incontinence, and they also discussed future directions to advance progress toward developing treatment strategies. Current therapies for the treatment of fecal incontinence were reviewed, including biofeedback, surgical repairs, and a gel, recently approved by the FDA, that is injected under the tissues surrounding the anus to tighten the anal opening.

A major theme of this meeting was the formidable challenge of raising the public awareness of this underdiagnosed condition. The panel raised concerns surrounding the lack of screening by health care providers, and the emotional and sociological difficulties that patients face in deciding to come forward and talk about this condition so they can get treatment. These hurdles were illustrated during a courageous testimony from a patient who shared her experiences living with fecal incontinence. Recommendations from this workshop will help guide future research on this debilitating condition.

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[1] *Whitehead WE, Borrud L, Goode PS, et al. Fecal incontinence in U.S. adults: epidemiology and risk factors. Gastroenterology. 137: 512–517, 2009.*

STORY OF DISCOVERY

Genetic Insights into Pancreatitis

Since the discovery in the 1990s of the first genetic risk factor associated with pancreatitis by an NIDDK grantee, subsequent studies supported by the Institute have identified a number of genetic variants associated with this disease. Pancreatitis is a disease marked by inflammation of the pancreas. A small gland located near the small intestine, the pancreas is responsible for producing enzymes that, mixed with bile from the gallbladder, aid in the digestion of food. In a healthy pancreas, these enzymes are released in an inactive form, to become activated only when they reach the intestine. However, when the pancreas is inflamed, as in pancreatitis, these enzymes become activated while still within the pancreas, where they degrade the very tissue that produced them, causing episodes of pain ranging from mild to severe, as well as nausea and vomiting. Pancreatitis can be acute, with inflammation resolving within a few days, or chronic, involving long-term inflammation and tissue damage. Over time, chronic pancreatitis leads to permanent damage to the pancreas and an increased risk of pancreatic cancer, one of the most devastating of all malignancies. Currently, there are no cures or preventive therapies for pancreatitis. But, investigations into how pancreatitis develops, including genetic variants that can contribute to this disease, have the potential to improve diagnosis, prevention, and treatment.

A variety of factors may contribute to the development of pancreatitis, often interacting in complex ways, including genetics, gallstones, heavy alcohol use, and other causes; unique combinations of factors may occur in different people. NIDDK-sponsored research has led to advances in the discovery of genetic factors associated with hereditary, chronic, acute, and other forms of pancreatitis. For example, the North American Pancreatic Study 2 (NAPS2) is a multi-center clinical study building on past research to uncover additional genetic markers that may help to identify individuals susceptible to pancreatitis and prevent the disease from developing. Genetic testing for mutations in pancreatitis-related genes based on this knowledge has the potential to provide information not only on disease risk, but also causes, severity, and likelihood of progression.

Following are a few highlights of NIDDK-sponsored research advances over the past few decades that have contributed to an explosion of new knowledge regarding the role of genetic factors in pancreatitis.

Trypsin-related Genetic Factors

In 1996, scientists identified the first pancreatitis-related gene mutation in patients with a rare genetic form of the disease called hereditary pancreatitis. This gene coded for the protein cationic trypsinogen, an inactive precursor form of the digestive enzyme trypsin. Trypsinogen is produced by cells in the pancreas then secreted into the small intestine, where it becomes activated to trypsin, an enzyme that breaks down protein and activates other digestive enzymes. Normally, the body has a fail-safe mechanism whereby trypsin is programmed to self-destruct if prematurely activated while still inside the pancreas. However, the mutations in the trypsinogen gene found in patients with hereditary pancreatitis disable this mechanism, enhancing trypsin activation in the pancreas, where it causes cell damage and pancreatitis.

Since that initial groundbreaking discovery, additional mutations associated with pancreatitis were identified in the trypsinogen gene, as well as in other genes that

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relate to trypsinogen/trypsin function. Mutations in the gene associated with cystic fibrosis—the *cystic fibrosis transmembrane conductance regulator* gene (*CFTR*)—were linked in 1998 to the pancreatitis of unknown cause that develops *in utero* in these patients. *CFTR* codes for an ion channel that plays an important role in maintaining the fluid that flushes enzymatic precursors like trypsinogen out of the pancreas and into the small intestine. In 2000, mutations in the *pancreatic secretory trypsin inhibitory* gene (*PST1/SPINK1*), which encodes a trypsin inhibitory protein, were identified in patients with pancreatitis that cause a loss of this inhibitory function, disabling this first line of defense against prematurely activated trypsin in the pancreas. Later, additional genes coding for proteins that affect trypsin activation, such as the *chymotrypsinogen C* gene and *calcium sensing receptor* gene, were also found to contribute to pancreatitis susceptibility.

New Genetic Variants

Recent findings of genetic studies on pancreatitis have pointed to other, non-trypsin-related mechanisms that offer insights into disease development and potential targets for prevention or therapy.

In 2012, the first genome-wide association study of pancreatitis identified a new gene associated with pancreatitis. Using samples collected by the NAPS2 Study from individuals with recurrent acute pancreatitis, chronic pancreatitis, or healthy controls, the researchers scanned their genomes to identify associations with variations in two genetic regions—one in the *trypsinogen* gene and another in a gene called *claudin-2*. Claudin-2 is a tight junction protein that regulates the movement of ions and water. The *claudin-2* gene variant was strongly associated with chronic pancreatitis, particularly in disease resulting from alcohol abuse in men. This finding could explain, in part, why most patients with alcohol-related pancreatitis are men.

Recently, researchers again used samples from the NAPS2 Study to identify mutations in a *gamma-glutamyltransferase 1* (*GGT1*) gene associated with chronic pancreatitis; these same mutations had been previously linked to pancreatic cancer. The protein formed from this gene typically maintains glutathione levels, which protect cells against damage by free radicals and oxidation. This discovery suggests another mechanism through which pancreatitis may occur—one which may lead not only to inflammation, but also to cancer formation in the pancreas.

Future Directions in Pancreatitis Research

The Institute continues to identify areas of new research opportunity related to pancreatitis, such as genetic risk factors, with help from stakeholders in the external research, professional, and patient advocacy communities.

In June 2012, the Institute convened a two-day research workshop at the NIH campus in Bethesda, Maryland, entitled "Advances in Acute and Chronic Pancreatitis: From Development to Inflammation and Repair." This workshop provided an update on a wide range of research efforts addressing acute and chronic pancreatitis, including susceptibility genes, and charted a course for advancing future research in this area by addressing current gaps and opportunities. Organizers included NIDDK staff, members of the NIDDK National Advisory Council who are active in this area, and the National Pancreas Foundation, as well as others in the external pancreatitis research community. Presenters from around the globe shared their findings, representing research institutions from the United States, England, Canada, Germany, and Spain. Participants also shared research resources, such as the "Pancreapedia" website, which is supported in part by the NIDDK (*www.pancreapedia.org*).

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The proceedings of this workshop continue to guide investigators toward promising future research directions in this area. Consistent with one of its objectives, a report summarizing the meeting was published in the January 2013 issue of the journal *Gastroenterology*, in order to share knowledge and recommendations with the larger research community focused on advancing understanding and improving care for those with acute and chronic pancreatitis. NIDDK staff also published an article summarizing gaps and opportunities in pancreatic disease research identified in this workshop in the May 2013 issue of the journal *Pancreas*, to reach a broader portion of the research community and encourage their applications for available funding mechanisms to boost research in this area.

In June 2013, the NIDDK and National Cancer Institute co-sponsored a two-day workshop at the NIH campus on "Pancreatitis, Diabetes, Pancreatic Cancer." The purpose of the workshop was to examine risk factors contributing to the development of pancreatic cancer, to address clinically relevant questions regarding treatment and surveillance of at-risk populations, and to identify opportunities and priorities for further research.

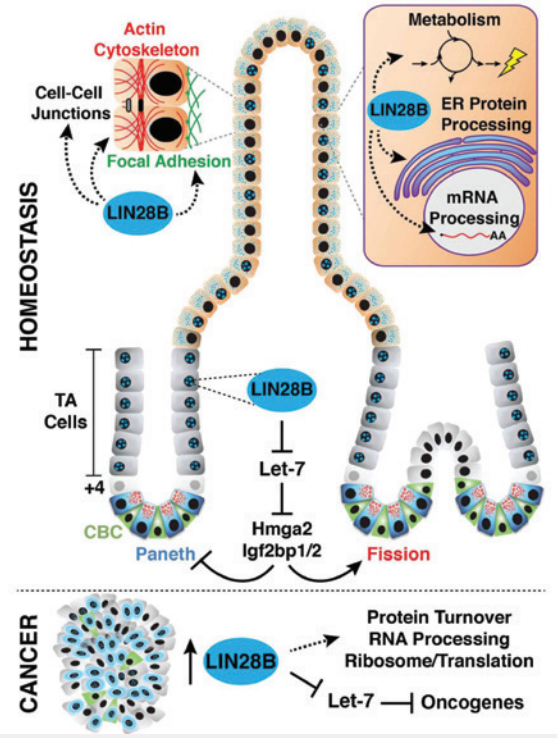
Through ongoing NIDDK studies of pancreatitis, such as NAPS2 and the Study of Nutrition in Acute Pancreatitis, as well as new efforts with its research partners, the story of genetic factors in pancreatitis is still being written. And, with this research, chances are steadily increasing for a happy ending where those at risk from pancreatitis are identified and the disease is effectively managed.

SCIENTIFIC PRESENTATION

The Yin and Yang of Intestinal Stem Cell Biology

Dr. Anil Rustgi

Dr. Anil Rustgi is the T. Grier Miller Professor of Medicine and Genetics and Chief of the Division of Gastroenterology at the University of Pennsylvania School of Medicine in Philadelphia, Pennsylvania. Dr. Rustgi earned his B.S. from Yale University and his M.D. from Duke University. Following a medical internship and residency at Beth Israel Hospital, Harvard Medical School, he completed his fellowship training in gastroenterology at Massachusetts General Hospital, Harvard Medical School, where he rose to the rank of Associate Professor of Medicine. He was Editor-in-Chief of the journal Gastroenterology *(2006-2011), Editor of the textbook* GI Cancers*, and Associate Editor of the textbook* Goldman's Cecil Medicine*. His research focuses on the molecular genetics of gastrointestinal epithelial cellular processes, including those originating from the esophagus, pancreas, and colon, as well as the roles of oncogenes, tumor suppressor genes, tumor initiation and progression, and tumor microenvironment. Dr. Rustgi's work is supported by both the NIDDK and the National Cancer Institute. His academic record includes over 200 publications and 100 book chapters, reviews, and editorials. He is also active in teaching and mentoring the next generation of biomedical researchers at the undergraduate, graduate, and medical school levels, with nearly 25 students and 30 fellows trained in his lab, 10 of whom now have their own independent and leadership positions. Dr. Rustgi presented his laboratory's recent research findings at the February 2013 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council. The following are highlights from his presentation.*



This diagram illustrates the complex interactions between molecules such as LIN28B and Let-7 in governing the activities of intestinal stem cells, which are located at the base of structures in the gut lining called "villi." These molecules can influence whether intestinal stem cells engage in everyday activities that maintain the healthy intestine (termed "homeostasis") or escape normal controls, leading to diseases such as cancer. ***Image courtesy of Dr. Anil Rustgi, adapted from an image from Madison, et al. LIN28B promotes growth and tumorigenesis of the intestinal epithelium via Let-7.*** ***Genes Dev*** ***October 15, 2013, 27: 2233-2245, http://genesdev.cshlp.org/content/27/20/2233.full, copyright 2013, Endocrine Society.***

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Every 5 to 6 days, an amazing feat of self-renewal takes place in the human gastrointestinal tract. Its inner lining, called the intestinal epithelium, completely rejuvenates itself—sloughing off old, damaged cells and replacing them with new ones that carry on the many important intestinal functions such as nutrient absorption and defense against pathogens. To create this new "inner you" repeatedly throughout the human lifespan, intestinal stem cells continually proliferate before "differentiating," or committing to becoming a particular type of intestinal epithelial cell. But, this same process of active proliferation and cell expansion can also have a dark side—intestinal epithelial renewal can go awry in disease states such as cancer, inflammatory bowel diseases, and infectious diarrhea, amongst others.

How do stem cells of the intestinal epithelium balance the light and dark aspects of their unique nature, proliferating just enough to replace worn-out cells without becoming "transformed" into tumors or contributing to other forms of gastrointestinal disease, and what happens at the cellular and molecular levels when this equilibrium is lost? Dr. Rustgi shared findings from his lab's explorations into some of the yin and yang elements of intestinal stem cell biology.

A Tale of Two Stem Cell Populations

The research summarized by Dr. Rustgi was supported in part through the Intestinal Stem Cell Consortium, a team of scientists from research centers across the country established in 2009 by the NIDDK and the National Institute of Allergy and Infectious Diseases to work together toward advancing understanding of intestinal epithelial stem cell biology during development, homeostasis, regeneration, and disease, as well as to aid the development of new therapies for intestinal diseases. Additional support for the research was provided through an NIDDK R01 grant to Dr. Rustgi and his team.

Dr. Rustgi began his story of the stem cells that replenish the intestinal epithelium with a *Fantastic Voyage*-style journey to the inner surface of the intestine, which is covered with fingerlike projections called villi. There at the base of the villi, within an unlikely structure for the birth of new cells called the "crypt," reside what scientists believed for many years was a single population of stem cells that gave rise to all the intestinal epithelial cell types with their unique functions, such as absorptive cells called enterocytes and several kinds of secretory cells. But, using a process that labels stem cells based on their gene products, recent studies by other groups have identified two distinct intestinal stem cell populations. These stem cell populations display different but complementary characteristics: one type proliferates under normal conditions, while the other typically lies dormant until injury or another form of stress triggers its proliferation. Intestinal stem cells are influenced by their local community of neighboring cells and the stroma, or stem cell "niche," as well as the molecular signals passing between them, which are altered by stressors such as infection, inflammation, and cancer. Scientists have also used this genetic technology to track the "daughter cells," or progeny of these stem cells, to better understand intestinal stem cells and their dual nature—their capacity to renew the intestinal epithelium, but also their potential to lead to disease. As part of these investigations, scientists in Dr. Rustgi's lab are focusing on two interdependent molecules that influence the stem cells' fate.

The Dark Side of Stem Cells

One molecule implicated in the intestinal stem cell's journey down the dark path of disease is one that under certain conditions can contribute to cancer; it is called *LIN28*. First discovered in *Caenorhabditis elegans*, a worm commonly used in molecular research, *LIN28* is also present in humans and comes in two forms—a and b. Normally, the LIN28 proteins

bind to some RNA molecules that encode proteins, and inhibit processing of certain other types of RNA molecules, called "microRNAs." MicroRNAs do not code for any proteins themselves, but exert their effects on the production of other RNAs, either by facilitating or inhibiting processing of some RNAs that do code for proteins. Through its interactions with RNAs, LIN28 affects glucose metabolism. It is also famous among scientists as one of several factors that, when added experimentally to adult somatic (nonreproductive) cells, can induce the formation of stem cells.

Dr. Rustgi described work led by Dr. Blair Madison, a Research Associate in the Rustgi lab and the University of Pennsylvania GI Division, who also has an NIDDK K01 grant. Dr. Madison and other scientists in Dr. Rustgi's lab used mouse models to explore the physiological impact of factors such as LIN28. For example, in a mouse model genetically engineered to activate the mouse form of Lin28b at varying levels only in cells of the intestinal epithelium, they observed expanding cell proliferation with high Lin28b levels. The mice also had overgrown or "hypertrophic" intestines and colons, developing colon polyps and then colon cancer as they aged. These results provided evidence supporting Lin28b's cancer-causing properties when present at high levels in the intestine.

However, Lin28b also showed an unexpected relationship to stem cell fate decision-making. Using a stain to visualize cells at the base of the crypts, mice with elevated Lin28b showed a loss of the secretory Paneth cells that interdigitate with the stem cells and release antimicrobial chemicals; however, no simultaneous loss of stem cells was observed. This finding challenged a prevailing theory that Paneth cells were necessary for supporting neighboring stem cells.

Balancing Act

What keeps pro-growth molecules such as LIN28 in check just enough to achieve continuous but controlled intestinal epithelial cell turnover? Balancing the effects of molecules such as LIN28 are forces such as so-called "tumor suppressors" that act as a molecular safety mechanism to balance pro-growth signals in the body. One such tumor suppressor is *let-7*. Also discovered in the *C. elegans* worm, *let-7* is a type of microRNA with the power to control certain genes involved in cell proliferation. *Let-7* is also targeted by LIN28, which inhibits *let-7* formation. LIN28 and *let-7*, therefore, represent opposing forces—LIN28 encourages cell proliferation, while *let-7* inhibits it. Under normal conditions, *let-7* helps keep intestinal stem cells from crossing over to the "dark side" of cancer by suppressing genes that control cell proliferation. However, when there is an overabundance of LIN28, it inhibits *let-7*, effectively taking the brakes off of the cell so that it races ahead with its proliferation program.

For example, when Dr. Madison on Dr. Rustgi's team generated and studied genetically modified mice that were missing *let-7* specifically in cells of the intestine and colon, they observed a rise in pro-growth genes, agreeing with *let-7*'s important role in slowing growth. The team next looked at mice that were genetically altered so that not only were they missing *let-7*, but they were also producing high levels of LIN28b. These mice showed a loss of the secretory Paneth cells along with some intestinal overgrowth or "hypertrophy"—signs that many, but not all, of LIN28b's effects are dependent on *let-7*. LIN28b likely targets other molecules besides *let-7* that are involved in the intestinal cell-growth balancing act.

SCIENTIFIC PRESENTATION

New Frontiers in Intestinal Stem Cell Biology
Researchers in Dr. Rustgi's lab are now pursuing the discovery of other molecular targets of LIN28b besides *let-7* using new RNA sequencing techniques that allow the identification of targets of RNA binding proteins. Surprisingly, most of the targets discovered to date by Dr. Rustgi's lab participate in pathways related to metabolism and protein complexes at cell connections, presenting opportunities for new lines of research into the relationship between these pathways and disease states such as cancer. Dr. Rustgi and his colleagues plan to continue their search for new insights into the complex opposing forces underlying intestinal stem cell biology, RNA processing pathways, and diseases of the gastrointestinal tract.

INTESTINAL STEM CELL CONSORTIUM

In 2009, the NIDDK teamed up with the National Institute of Allergy and Infectious Diseases (NIAID) to establish the Intestinal Stem Cell Consortium. This Consortium brings together a group of investigators from research centers across the country to collaborate on a common mission: to advance understanding of stem cell biology in the intestinal epithelium and its role in organ development, homeostasis, regeneration, and disease, in order to provide a foundation for the development of new therapies for digestive diseases. The Consortium grew out of a March 2008 workshop hosted by the NIDDK on "Local Influences on Health and Repair of Intestinal Epithelium" and also aligns with multiple goals in the March 2009 research plan of the National Commission on Digestive Diseases. To date, Consortium support has resulted in the publication of 28 original scientific articles and counting. These research advances offer fresh insights into intestinal stem cell biology, as well as highlight new research methods and resources to facilitate future progress in this field.

In 2013, the NIDDK and NIAID announced an initiative to continue this productive Consortium. As part of the renewed Consortium, scientific exchange will be maximized and research accelerated through sharing of information, data, biomaterials, models, reagents, resources, and methods within the Consortium and with the larger research community. A coordinating center will facilitate the Consortium's activities and communication of research results, data, and methods amongst members and with the community. The Consortium will continue to support ongoing studies to isolate and characterize intestinal stem cells, compare stem cell populations, and determine how their activity is controlled. While the Consortium emphasizes research on stem cells of the small intestine, methods and research results stemming from these projects are expected to aid similar studies relevant to other areas of the gastrointestinal tract and other organs.

Additional information about research supported by the Intestinal Stem Cell Consortium can be found on its website at: http://iscc.coh.org/

PATIENT PROFILE

Nancy Norton
Living Hour to Hour with Fecal Incontinence


Nancy Norton

On many days, Nancy Norton can only plan her life hour by hour. When she was 35, while giving birth to her son, Nancy experienced a fourth degree perineal tear (a tear extending from the vagina to the rectum). The injury included damage to the anal sphincter muscles, which play a critical role in controlling bowel evacuations. As a result, for the past 28 years she has lived with fecal incontinence, which is the accidental passing of solid or liquid stool or mucus from the rectum.

Nancy's incontinence was revealed when, shortly after giving birth, she was hosting friends and family at her home in Milwaukee to see her new baby. "I was sitting in a chair, and I just stood up and had stool running down my leg," she recalls. Like many women who have experienced similar injuries, Nancy was caught completely off guard. Mortified and alarmed, she contacted her doctor immediately, telling herself, "I need to find out what's going on here."

When Nancy first contacted her health care provider, the response she received conveyed a practically dismissive tone. She was told there was a flu going around, and perhaps she just had an upset stomach. While Nancy felt her doctors were seeing her incontinence as little more than an inconvenience, she was starting to suspect that it was something more serious than the flu. When the condition did not improve, she underwent two rounds of surgery with the hope that repairing the injured tissue would be enough to resolve her incontinence. The first repair broke down, however, and the second repair was not successful. Nancy gradually began to realize that she might be facing a long battle with incontinence. "After waking up for months, hoping that this day was going to be the day when everything was going to work right," she remembers, "it starts to sink in that this is not changing." With no easy answers, Nancy felt stranded. "I was like a lot of other people, where it was a result of an obstetrical injury, and you just automatically assume that it can be fixed. But when the attempts to fix it don't work, then what do you do?"

"After waking up for months, hoping that this day was going to be the day when everything was going to work right," says Nancy, "it starts to sink in that this is not changing."

That time was especially challenging for Nancy because she had also just become a mother. "Here's this new little baby," she says, remembering those tough first days of parenthood, "and I was trying to be

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a mother while going back in the hospital for surgery. It was overwhelming." She faced juggling many major changes in her lifestyle at once: being a new parent, changing her diet, trying always to be near a bathroom, and bringing a bag of extra clothes and cleaning materials whenever she went out. "I was learning how to live with this new body, and being a new mom, and caring for the needs of my son," recalls Nancy. As a result of her experiences, she now understands the importance of not only addressing the medical issues associated with fecal incontinence, but also thinking about its effects on family and personal life.

Fecal Incontinence: A Hidden Condition

Nearly 18 million U.S. adults—about 1 in 12—have fecal incontinence, commonly referred to as bowel control problems. The high prevalence of this condition may seem surprising, but this is probably because most people who have fecal incontinence are reluctant to talk about it, and health care providers rarely screen for it, so it is significantly underdiagnosed. Besides feeling too embarrassed to discuss episodes of fecal incontinence, patients are also mired by the suspicion that their health care providers will not be able to help them, or they may just despondently accept the condition as a "normal" part of aging.

While fecal incontinence is more common in older adults, it can affect people of any age. Several factors can increase the risk of a bowel control problem, including: diarrhea; urgency, or the sensation of having very little time to get to the toilet for a bowel movement; a disease or injury that damages the nervous system; injury to the pelvic floor (the muscles, ligaments, and tissues that support the uterus, vagina, bladder, and rectum), which may occur during a difficult childbirth; and poor overall health from multiple chronic (long-lasting) illnesses. Other risk factors include depression, being physically inactive or overweight, or having type 2 diabetes.

Regardless of the cause, a serious case of fecal incontinence means a life that revolves around being near a bathroom, Nancy explains. This might mean only being able to make plans for the next hour or two at a time, which has the tendency to feel as if the incontinence is controlling a person's daily life. "Most people don't even think about the process of defecation, but you end up focusing almost 24/7 on controlling your bowels," she says. To make matters worse, accidental bowel movements carry such a social stigma that, rather than seeking help, many people with fecal incontinence painstakingly attempt to hide their symptoms with accessories like absorbent padding and deodorant sprays, or they try to manage the condition through changes in their diet that are not always healthy. They especially become very conscious of how much food and water they are taking in—sometimes to drastic levels. "A lot of incontinent people will think, 'If nothing goes in, nothing will come out,'" Nancy explains. "Well, that's not a healthy way to live. But it's those kinds of things that naturally go through your mind to try to control fecal incontinence."

"Most people don't even think about the process of defecation, but you end up focusing almost 24/7 on controlling your bowels," Nancy says.

Instead of leading a secret life, Nancy chose to keep looking for answers. After her surgical repairs failed to cure her incontinence, she set off to find doctors at some of the country's best medical institutions who may have other ideas for treatments. Some suggested more surgery, which Nancy was reluctant to try; others suggested a less familiar type of therapy called "biofeedback." Biofeedback, which teaches patients how to control bodily functions, is used for a number of conditions; for fecal incontinence, it is designed to educate people on the muscles that

control bowel evacuations. It improves the awareness of sensations in the lower intestine by using tools such as small inflatable balloons in the rectum, which assist in teaching people with fecal incontinence to coordinate the sensation of rectal filling with squeezing of the external sphincter muscle. Another aspect of biofeedback involves special sensors which measure bodily functions and deliver information to health care providers, who use the readings to help their patients make beneficial changes. Research sponsored by the NIDDK has provided encouraging results supporting biofeedback as an effective method for treating fecal incontinence.

Biofeedback helped Nancy regain some control over her daily life. "Biofeedback really helps you understand the physiology of how things work, about muscle groups and coordination of those muscle groups, so even though I am still incontinent, I feel like I have some kind of control," she says. It helped her learn how to be prepared and to think about what she needs to take with her during the course of the day. Even then, adjustment back to a daily routine required small steps. She would start by leaving her home for just a short period of time. She would ask herself, "could I be away from the house for an hour comfortably without being near a bathroom?" She forced herself to take a class at a university because it put her in a social situation where it would be difficult to be able to get up and leave. She made an effort to "get back out there and do things" that wouldn't result in her being overcome with anxiety, which can raise the sense of needing to be near a bathroom. To be out in public and have access to a restroom is not always easy, says Nancy, but "as an incontinent person, you learn how to navigate through life in a different way."

Advocating for Others

As a result of her experiences with fecal incontinence, her struggles with finding proper care, and her continuing journey through life with the condition, Nancy founded the International Foundation for Functional Gastrointestinal Disorders (IFFGD), a nonprofit education and research organization that helps people affected by gastrointestinal disorders. "I thought, I can't be the only one who is having these kinds of issues," she says. After IFFGD was established, "we had people calling us with every gastrointestinal condition you can think of because there was no other place for them to go." Since its start in 1991, the IFFGD has been a source of educational information, support, and assistance for people who suffer with functional gastrointestinal and motility disorders, including fecal incontinence and irritable bowel syndrome.

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Even with a resource like the IFFGD, raising awareness of fecal incontinence is not an easy task, primarily because of the negative public perceptions of the condition. In a sense, fecal incontinence is a hidden disability, because the anxiety associated with it is an enormous burden, yet people tend to suffer in silence, or they rarely leave their homes. Travelling becomes an even bigger problem. Nancy knows "the anxiety of not being able to get out of your seat for the last half an hour [of a flight], and you just hope that everything is fine. Or checking into a hotel room early because you want to get into your own room and have your own bathroom." Even taking care of simple errands like going to the grocery store becomes an exercise in preparedness, as people with fecal incontinence

must be constantly aware of where the restrooms are. Even then, they must bring extra supplies, such as incontinence wipes, because public restrooms are ill-equipped to help someone clean themselves after an episode of incontinence.

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The fear of having an accidental bowel movement in public can also be debilitating to someone's social life, especially if they are too embarrassed to explain why they cannot attend a gathering. Nancy is all too familiar with the questions that go through the mind of a person with fecal incontinence when asked to go out: "Do I feel comfortable enough to explain that I may not be able to make it? I might not be able to go to church, or sign up to volunteer for something; am I comfortable enough in explaining why I'm not there?" For the most part, says Nancy, people are understanding, but they do not necessarily comprehend the nature of her condition. People with fecal incontinence, she says, have to decide: "Are we going to venture out? How do we plan our day? What are we going to need? How are we going to handle [the incontinence]?" When accidents do happen, they can be psychologically crippling. "It's a very emotional experience when it happens, particularly in public," says Nancy about fecal incontinence. "It's enough to stop a person in his tracks, and then you don't want to go back out there again. You don't want to put yourself in that situation, because society is not always kind to people who are incontinent."

Hope Through Research and Awareness

Nancy believes that perception of fecal incontinence has improved slightly since her diagnosis almost 3 decades ago, but there is still much work to be done to raise awareness among medical caregivers and the public. Since 2011, the NIDDK has managed the Bowel Control Awareness Campaign, which provides current, science-based information about the symptoms, diagnosis, and treatment of fecal incontinence, including resources such as advice for talking to doctors and links to professional and voluntary organizations for help and support. In August of 2013, the NIDDK hosted a workshop entitled "Developing a Clinical Research Agenda for Fecal Incontinence," where a panel of experts discussed major issues in the diagnosis and treatment of fecal incontinence, and future research directions. There were also discussions about raising public awareness of fecal incontinence to make it easier for patients with the condition to come forward and seek treatment.

Nancy continues to live with fecal incontinence. But, as an advocate for millions of people who are affected by a largely secret condition that seizes control of their daily lives, she has shown the courage to confront negative perceptions and inspire hope in what many would see as a hopelessly distressing situation. She credits her biofeedback therapist and a strong network of people close to her as vital components to help her cope with incontinence. She says her husband, a co-founder of IFFGD, "has been by my side from the beginning, never wavering, always there to help me every day." She also has a group of supportive friends who understand her difficulties with social gatherings. "They're always saying things like, 'What can we all do that Nancy can do?' And that really means a lot to me. And I have been very thankful." She adds, "But this is something that is not going away, and there are a lot of people who really need help… [They are] living with fecal incontinence and are really doing the best they can to manage it."